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Prevalence of *Escherichia coli* Strains Exhibiting Genetic Recombination

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The first bacterium to be tested by an efficient selective method for the occurrence of genetic recombination was strain K-12 of *Escherichia coli*. Experiments with auxotroph mutants of this strain promptly gave conclusive, positive evidence of genetic exchanges between different mutant cells in mixed cultures (*1, 2*). However, subsequent attempts to obtain comparable results with a number of other strains used for genetic work were fruitless.

Cavalli and Heslot (*3*) examined a number of auxotroph strains from the National Type Culture Collection (England) and found one that could be crossed with K-12. Unfortunately, this isolate has a complex nutrition, so far unanalyzed, which greatly hinders further work. In other characteristics it closely resembles K-12.

It would be surprising if K-12, the first *E. coli* strain examined, should prove to be uniquely suitable for crossing experiments. Unfortunately, the method for testing fertility involved a good deal of work: it was necessary to prepare at least two nonoverlapping, double nutritional mutants from each strain. Despite improved techniques (*4*), such a procedure is almost prohibitive for routine survey of new strains. The following procedure was therefore put into effect for preliminary screening.

A multiple marker strain, W-1177 (= 677-sr in [*5*]) has been developed from K-12 by a long sequence of mutational steps. This strain differs from the wild-type strain K-12 in these markers: polyauxotrophy for threonine, leucine, thiamin; resistance to streptomycin and to bacteriophage T1; failure to ferment lactose, maltose, mannitol, xylose, galactose, or L-arabinose. These may be symbolized as: $T-L-B_1-S^r$ V_1^r $Lac-Mal-$ etc. Typical wild-type *E. coli* strains are $T+L+B_1+S^s$. These four markers are useful in detecting recombination between W-1177 and new strains to be screened. Heavy inocula of W-1177 and of the propositus are mixed in a complete broth tube, and incubated for 6–24 hr. The mixed culture is then harvested, and the washed cells are plated on a minimal agar medium containing 100–1,000 μg/ml streptomycin. The minimal agar selects prototroph cells; the streptomycin selects S^r. The minimal streptomycin agar thus permits the growth only of $T+L+B_1+S^r$ colonies and suppresses the two parents. This assortment of characters can arise either by recombination, or by mutation of the propositus from S^s to S^r. Fortunately, this mutation occurs at an extremely low rate, about once per 10^{10} cell divisions (*6*), and therefore confusion between recombinants and mutants is minimized. On the other hand, the improbable coincidence of three reverse mutations needed to produce a prototroph from W-1177 has never been observed in extensive controls (*1, 2*).

The principal function of the screening procedure is the rational selection of cultures appropriate for more detailed analysis by the development of auxotroph mutants. Even in this preliminary test, however, recombination of unselected markers (V_1, *Lac, Mal,* etc.) among the S^r prototroph selections usually verified the occurrence of genetic interchange.

Two groups of cultures have been screened so far for cross-fertility with W-1177 (i.e., K-12). About 40 cultures from chicken cecal flora (supplied by courtesy of S. Shapiro) yielded one isolate that crosses, but very poorly. About 100 isolations from human urine cultures (secured through courtesy of the Wisconsin State Laboratory of Hygiene) have given 8 that cross with about the same facility as K-12, and an equal number that appear to be less fertile (if fertile at all), so that the evidence for recombination in the latter is still inconclusive. The possibility that some ecotypic differentiation is revealed by the breeding test deserves further study when it is recalled that K-12 is also of human origin.

Nutritional mutants are being prepared in the new isolates. The three cultures so far tested cross freely with each other, as well as with K-12 and within each strain.

The new strains differ in a number of characteristics, including fermentation patterns (3 are sucrose-positive; 6, sucrose negative; one is a lactose-negative "paracolon" type), colony morphology (*R, S,* and intermediates by the acriflavine test), and patterns of resistance to and production of colicins (*7*) and phages. Preliminary serological studies are under way, in addition to experiments to uncover cryptic genetic differentiation. There is a strong suggestion that colicin and lysogenicity interactions may act as genetic isolation mechanisms.

Unfortunately, the survey method does not reveal other intrafertile, intersterile breeding groups, nor, owing to the dominance of S^s (*8*), can it reveal unreduced diploid hybrids between the different strains. Despite these shortcomings, however, the streptomycin-prototrophy selection method has succeeded in displacing strain K-12 from its position as the only "sexual" bacterium.

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